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RNA editing of the serotonin 2C receptor and expression of Gα_q protein: genetic mouse models do not support a role for regulation or compensation

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Abstract

The serotonin 2C (5-HT$_{2C}$) receptor undergoes RNA editing at five bases in a region of the pre-mRNA encoding the second intracellular loop, generating many unique 5-HT$_{2C}$ receptor isoforms. Mechanisms regulating *in vivo* expression of different edited 5-HT$_{2C}$ receptor isoforms are poorly understood, as are the adaptive consequences of variation in editing profiles. Recent findings suggest a putative relationship between expression levels of G$\alpha_{q/11}$ protein and the degree of editing of 5-HT$_{2C}$ receptor transcripts. To elucidate the potential regulatory or adaptive role of G$\alpha_{q/11}$ protein levels, we quantified editing of 5-HT$_{2C}$ receptor RNA transcripts in Gα_q null mice and protein levels of Gα_q and Gα_{11} in transgenic male mice solely expressing either the non-edited (INI) or the fully edited (VGV) isoforms of the 5-HT$_{2C}$ receptor. Pyrosequencing of RNA isolated from amygdaloid cortex in Gα_q null and wild-type mice revealed no significant differences in 5-HT$_{2C}$ receptor mRNA editing profiles. Cortical tissue from INI/y, VGV/y, and wild-type mice was assayed for expression of Gα_q and Gα_{11} subunits by Western blotting. No differences in signal density between wild-type and INI/y or VGV/y groups were found, indicating equivalent levels of Gα_q and Gα_{11} protein. Together, these data do not support a causal or compensatory relationship between 5-HT$_{2C}$ receptor RNA editing and G$_q$ protein levels.

Keywords

mRNA editing; serotonin 2C receptors; INI; VGV; Gα_q; Gα_{11}

1. Introduction

The serotonin 2C (5-HT$_{2C}$) receptor is expressed in several brain regions, including the cortex, choroid plexus, striatum, hypothalamus, and amygdala, (Lopez-Gimenez *et al.* 2002, Abramowski *et al.* 1995). Consistent with this distribution, 5-HT$_{2C}$ receptors are involved in regulating behaviors including feeding, fine motor movements, and emotional states, and are a target for pharmacological treatment of related disorders (Millan 2005, Di Giovanni *et al.*

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Disclosure/Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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2006, Jenck *et al.* 1994, Lam *et al.* 2007, Dunlop *et al.* 2006, Nilsson 2006). 5-HT_{2C} receptors are coupled to several G-protein α-subunits, including $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{12}$, $G\alpha_{13}$, and $G\alpha_{i/o}$ (Bockaert *et al.* 2006, Cussac *et al.* 2002, McGrew *et al.* 2002, Price *et al.* 2001). This expansive range of coupling allows the 5-HT_{2C} receptor to modulate functioning of several intracellular signaling pathways. One of the most thoroughly characterized signal transduction pathways modulated by the 5-HT_{2C} receptor is the $G\alpha_{q/11}$ phospholipase Cβ (PLCβ) pathway (Conn *et al.* 1986, Qiu *et al.* 2007). PLCβ stimulates hydrolysis of phosphatidylinositol (4,5)-bis-phosphate (PIP2), producing both inositol triphosphate (IP3), that mobilizes intracellular Ca^{++}, and diacylglycerol (DAG) that consequently activates protein kinase C.

Potentially 24 different protein isoforms of the 5-HT_{2C} receptor derived from 32 possible RNA isoforms are generated by RNA editing, an enzymatic process that converts adenosine moieties to inosine at five sites, termed A,B,C,D, E, in the 5-HT_{2C} receptor RNAs. RNA editing at all sites yields the codons for valine (V), glycine (G), and valine (V) to generate the fully-edited (VGV) isoform, while an absence of editing at all sites yields the codons for isoleucine (I), asparagine (N), and isoleucine (I), to generate the non-edited (INI) isoform. *In vitro* assays show that the non-edited isoform has high constitutive (agonist-independent) and agonist-stimulated activity compared to the more highly edited VSV or VGV isoforms. These observations suggest that differentially edited 5-HT_{2C} receptor isoforms may couple either more or less efficiently to G-proteins and thus may have unique signal transduction signatures *in vivo* (Berg *et al.* 2001, Burns *et al.* 1997, Fitzgerald *et al.* 1999, Berg *et al.* 2007, Niswender *et al.* 1999, Berg *et al.* 2008).

Mechanisms controlling *in vivo* expression of different RNA edited 5-HT_{2C} receptor isoforms are poorly understood, but behavioral stress appears to alter the relative frequency of 5-HT_{2C} receptor editing in both mouse and rat rodent models (Iwamoto *et al.* 2005b, Englander *et al.* 2005, Bhansali *et al.* 2007). In mice, the stress of infant maternal separation leads to an increase in 5-HT_{2C} receptor editing accompanied by an increase in $G\alpha_q$ transcripts and protein in cerebral cortex (Bhansali et al., 2007). This experimental association between altered $G\alpha_q$ expression and changes in 5-HT_{2C} receptor editing could be direct or spurious, as both increases in $G\alpha_q$ expression and 5-HT_{2C} receptor editing were a consequence of the manipulation; neither was an independent factor. Because G-protein coupling is less efficient for highly edited isoforms, increased G-protein could be an adaptation to maintain homeostatic signaling through 5-HT_{2C} receptors (Bhansali et al., 2007). Alternatively, increased G protein expression could elicit compensatory changes in 5-HT_{2C} receptor RNA editing to form transcripts with reduced coupling efficiency (Bhansali et al., 2007).

To elucidate the potential regulatory or adaptive role of $G\alpha_{q/11}$ protein levels and the extent of 5-HT_{2C} receptor editing, we examined the profile of 5-HT_{2C} receptor editing in transgenic mice in which the expression of $G\alpha_q$ is selectively ablated (Offermanns *et al.* 1997), and we examined the expression of $G\alpha_q$ and $G\alpha_{11}$in the cortex of transgenic mice solely expressing the non-edited (INI) or the fully edited (VGV) isoform of the 5-HT_{2C} receptor. We hypothesized that if G-protein expression is under the control of 5-HT_{2C} receptor editing, changes in G-protein levels would be evident in hemizygous mutant males bearing non-edited (INI/y) or fully-edited (VGV/y) alleles. Furthermore, if RNA editing of the 5-HT_{2C} receptor is modulated by G-protein expression, $G\alpha_q$ null mice would be expected to show an altered profile of edited 5-HT_{2C} RNA relative to wild-type litter mates.

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2. Materials and Methods

2.1. Animals

Adult male mice 2 to 3 months of age were used for all experiments. INI/y mutant mice on a 129S6 background were created by introducing multiple mutations into intron 5 to disrupt base-pairing with exon 5 and prevent RNA editing (Jacobs & Emeson 2006). VGV/y mutant mice were generated by introducing guanosine residues instead of adenosine at all five edited sites (Morabito *et al.* 2007). Analysis of 5-HT_{2C} RNA isolated from INI/y and VGV/y mice confirmed that only the INI and VGV isoform, respectively, was expressed. Hemizygous mutant males, INI/y or VGV/y, and wild-type (WT) male offspring, x/y, were used for Western blotting analyses.

The generation of transgenic $G\alpha_q$ null (−/−) mice has been described previously (Offermanns *et al.* 1997). For the present experiments, heterozygous [$G\alpha_q$ (+/−)] males and heterozygous females of a C57BL/6×129S6 background were mated to generate litters of wild-type and null mice. The genotypes of all mice were determined by PCR analysis of genomic DNA from tail samples as previously described (Offermanns et al. 1997). Animals had free access to food and water, and were maintained on a 12:12 h light/dark cycle (lights on at 0700 h). All experiments were done in compliance with the guide *Principles of Laboratory Animal Care* (NIH publication No. 85-23) and the Vanderbilt University Animal Care and Use Committee.

2.2. Pyrosequencing

Procedures to quantify RNA editing of 5-HT_{2C} receptor transcripts by pyrosequencing have been published previously (Sodhi *et al.* 2005, Hackler *et al.* 2006, Iwamoto *et al.* 2005a). Pyrosequencing is an established technology platform for short-read sequencing. It provides complete resolution of 5-HT_{2C} receptor transcripts (Sodhi *et al.*, 2005) and excellent one-to-one correspondence with long-read sequencing methods if the same bacterial colonies are sequenced (e.g., out of 93 colonies sequenced, 82 were common successes, 4 were unique pyrosequencing failures, and 7 were common pyrosequencing and sequencing failures; data available from David C. Airey).

Six male $G\alpha_q$ null (−/−) mice and six WT (+/+) litter mates were used to quantify editing of 5-HT_{2C} receptor transcripts. Mice were briefly exposed to isoflurane anesthesia, and then euthanized by cervical dislocation and decapitation. Brains were rapidly removed, and amygdala plus overlying cortex ventral to the rhinal sulcus was dissected from 2 mm thick coronal sections. Brain samples were homogenized in TriReagent (Sigma-Aldrich) followed by total RNA extraction using standard procedures. RNA concentration was measured by optical absorbance (NanoDrop Technologies, Inc). Two micrograms of total RNA was utilized for RT-PCR, with the following intron spanning primers, forward (sense) primer: 5' TTT CAA CTG CGT CCA TCA TGC ACC T 3'; reverse (antisense) primer : 5' AAC GAA GTT CGG GTC ATT GAG CAC 3'. Electrophoresis of the PCR products was performed using a 2% agarose gel. The 236 bp amplicon was excised and extracted with a QIAquick Gel Extraction Kit (Qiagen). PCR products were ligated and cloned using the TOPO TA Cloning for Sequencing kit (Invitrogen). Individual bacterial colonies were picked and lysed at 98°C for 8 minutes in PCR tubes containing sterile water and then kept at 4°C until use. An additional PCR amplification step was performed to generate a 110 bp biotinylated product using (per well): 0.1 μM forward primer: 5' TTT CAA CTG CGT CCA TCA TGC ACC T 3', 0.1 μM reverse primer: Biotin- 5' CGA ATT GAA CCG GCT ATG CT 3', 2.0 mM $MgCl_2$, 1.25 U AmpliTaq Gold DNA Polymerase (Applied Biosystems), 0.3 mMdNTPs, 1X PCR buffer, and 5 μl bacterial DNA lysate. Amplification was performed for 32 cycles using the following parameters: 96°C for 5 min, 96°C for 20 sec, 58°C for 30

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sec, 72°C for 20 sec, repeat steps 2–4 31 times, 4°C hold. Five microliters of PCR product from a random set of 8 wells was resolved on a 2% agarose gel to check quality. Pyrosequencing (www.biotage.com) was performed using 10 μM of forward sequencing primer, 5' ATA TCG CTG GAC CGG TAT GTA G 3', and the dispensation sequence, GCA GCT AGT CGT AGA GTC TAG CT.

To provide adequate precision, an average of 60 RNAs was sequenced per animal (717 successful pyrograms in total). Pyrograms were scored at the editing sites A, B, E, C, and D as a string of five zeros (non-edited) and ones (edited), e.g., "11010" is edited at A, B, and C. These data were re-coded to several binary dependent variables using a custom Stata script (www.stata.com, available from David C. Airey) based on the mapping between editing site and predicted amino acids shown in Table 1. Dependent variables included editing at individual sites (e.g., C), RNA isoform type (e.g., ABCD), and predicted receptor isoform (e.g., VSV). Percentage summary statistics were then calculated for each individual mouse, and genotype differences were tested by independent samples t-test. Tests were run for each edit site (A, B, E, C, and D), RNA isoform and predicted receptor isoform with at least 1% frequency (RNA isoforms = A, AB, ABC, ABCD, ABD, AD, D, U=non-edited; receptor isoforms = INI, INV, VNI, VNV, VSI, VSV). Multiple test correction by the Bonferroni procedure was considered for the number of tests performed across edit sites and RNA isoforms (13 tests), but not receptor isoforms (significance criterion = $0.05 / 13 < 0.004$).

2.3. Western Blotting of Cortical Tissue

Following brief isoflurane anesthesia and cervical dislocation, brains from mice were quickly removed. Cerebral cortex posterior to the frontal cortex was dissected on an ice cold dish, and quickly weighed and placed in a volume depending on weight of cold 20 mM phenylmethanesulfonyl fluoride, and membrane buffer solution containing 15mM Tris, 250 mM sucrose, 15 mM KCl, 15 mM NaCl, 6 mM disodium EDTA, 5 mM disodium EGTA, 50 mM NaF, 2 mM $Na_4P_2O_7$, 1 mM dithiothreitol, 0.2% Triton X and a cocktail of protease inhibitors (Complete, Roche Diagnostics, Germany). Tissue was homogenized using a sonicator, then centrifuged for 20 min at 11,337 x g at 4° C, and the supernatant was used for analyses. A Pierce (Thermo Scientific, USA) BCA Protein Assay kit was used to prepare samples of the supernatant for measurement of protein levels, and a NanoDrop (NanoDrop Technologies, Inc.) spectrophotometer was used to measure protein levels. The methods for this protein assay have been described previously (Lovrien & Matulis 1998). 30 μg of protein was resolved on 10% acrylamide gels and transferred to PVDF membranes (Amersham Hybond-P, G.E. Healthcare, U.K.) overnight with 17V constant, at 4° C. Immunostaining involved membrane washes in Tris-buffered saline, blocking with 4% nonfat dried milk, incubation with a monoclonal mouse α-tubulin antibody (1:7500, Sigma, USA) and a polyclonal rabbit $G\alpha_q$ antibody (1:500, Santa Cruz Biotechnology) or rabbit $G\alpha_{11}$ antibody (1:500, Santa Cruz Biotechnology, USA), followed by incubation with goat anti-mouse IgG-horse radish peroxidase (HRP, 1:20,000) (Sigma, USA) and goat anti-rabbit IgG HRP (1:7500, Millipore, USA). α-tubulin and $G\alpha_q$ or $G\alpha_{11}$ bands were detected using chemiluminescence (GE Healthcare, UK), with Amersham Hyperfilm ECL (GE Healthcare, UK). The experiment used cerebral cortex from 5 INI/y and 6 wild-type litter mates, 6 VGV/y and 6 wild-type litter mates, and 2 $G\alpha_q$ null mice. Densitometry of bands was performed using NIH Image J 1.39 (Abramoff *et al.* 2004).

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3. Results

3.1. Pre-mRNA editing profiles in $G\alpha_q$null mice

RNA editing of 5-HT_{2C} receptors from mouse amygdala and overlying cortex showed a very similar profile between male $G\alpha_q$ null mice and $G\alpha_q$ WT litter mates (Figure 1). A total of 22 RNA isoforms were observed, corresponding to 15 predicted receptor isoforms; of these, 8 RNA isoforms and 6 protein isoforms were not rare (> 1 %). Differences in editing efficiencies at sites A, B, E, C, and D were not greater than 4 percentage points (Table 2). Statistical tests suggest these differences most likely arose by chance, and none of the test probabilities were significant after correction for multiple tests. Similarly, none of the differences in any of the RNA isoforms were significant after multiple test correction; the largest difference was a 5% increase in the RNA isoform ABC in $G\alpha_q$ null mice (Table 3). Because there is a degenerate relation between the 32 possible RNA isoforms and the 24 predicted protein isoforms, the frequency of receptor isoforms was also inspected (Table 4). However, none of the comparisons of the predicted receptor isoforms provide any additional significant differences; the 5% VSI difference is expected from the ABC RNA isoform difference, given that AC isoforms were rare.

3.2. Gαq/11 proteins in transgenic mice

Representative Western blots are shown in Figure 2. $G\alpha_q$ or $G\alpha_{11}$ bands were seen at approximately 45 kDa; α-tubulin detection (a band at 50 kDa) was used as a loading control. Western blots from $G\alpha_q$ null mice showed α-tubulin and $G\alpha_{11}$, but no detectable $G\alpha_q$, confirming that the $G\alpha_q$ antibody was specific (Figure 2). The mean optical density ratio of bands corresponding to α-tubulin and $G\alpha_q$ or $G\alpha_{11}$ are plotted in Figure 3. An unpaired t-test of the ratio of $G\alpha_{11}$ to α-tubulin or $G\alpha_q$ to α-tubulin between INI/y mice and wild-type litter mates revealed no significant differences ($p = 0.94$ and $p = 0.72$, respectively). Similarly, the ratio of $G\alpha_{11}$ to α-tubulin or $G\alpha_q$ to α-tubulin between VGV/y mice and wild-type litter mates did not differ ($p = 0.77$ and $p = 0.56$, respectively)

4. Discussion

RNA editing alters the coding potential of the 5-HT_{2C} receptor to generate as many as 24 different protein isoforms. *In vitro* experiments document that edited isoforms of 5-HT_{2C} receptor, relative to the non-edited 5-$HT_{2C\text{-}INI}$ receptor, have reduced spontaneous $G\alpha_{q/11}$ protein coupling, and show a rightward shift in the dose-response curve for serotonin stimulated inositol phosphate production and arachidonic acid release, suggesting reduced serotonin potency in edited 5-HT_{2C} receptors (Price et al. 2001, Berg et al. 2008, Niswender et al. 1999, Herrick-Davis *et al.* 1999). Given these observations, it seems likely that mechanisms would exist *in vivo* to maintain homeostatic signaling if RNA editing of 5-HT_{2C} receptor occurs. Given the paucity of information about how 5-HT_{2C} receptor signaling is regulated *in vivo*, tests were conducted to determine whether changes in RNA editing lead to compensatory alterations in $G\alpha_{q/11}$ protein levels and, vice versa, if alterations in $G\alpha_q$ protein modify RNA editing profiles.

The relationship between the relative frequency of 5-HT_{2C} receptor RNA editing and steady-state levels of $G\alpha_q$ expression was addressed by measuring $G\alpha_q$ protein levels in cerebral cortex of mice solely expressing either the non-edited (INI) or fully-edited (VGV) 5-HT_{2C} receptor isoforms. Compared to their respective WT litter mates, transgenic INI/y or VGV/y mice did not show alterations in the levels of $G\alpha_q$ or $G\alpha_{11}$ proteins. In a prior study (Berg *et al.* 1999) it was suggested that 5-HT_{2C} receptor inverse agonists increase coupling of 5-HT_{2C} receptor to the $G\alpha_q$ PLCβ pathway by increasing expression of $G\alpha_q$ proteins. However, additional *in vitro* studies found no relationship between enhanced responsiveness

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of non-edited 5-HT_{2C} receptors and expression levels of $G\alpha_q$ protein (Chanrion *et al.* 2008). Also, increased high affinity binding of the 5-$HT_{2C/2A}$ receptor agonist DOI (2,5-dimethoxy-4-iodoamphetamine) in the hypothalamus after chronic fluoxetine treatment was not associated with increased levels of $G\alpha_{q/11}$ proteins (Li *et al.* 1997). These observations support the view that changes in coupling between the 5-HT_{2C} receptor and $G\alpha_{q/11}$ proteins occur in the absence of changes in the levels of $G\alpha_{q/11}$ proteins. Although it remains a possibility that compensatory changes exist *in vivo* to maintain homeostatic signaling through differentially edited 5-HT_{2C} receptor isoforms, the present findings do not support a role for variation in the expression of $G\alpha_q$ in the mouse cortex in this adaptive process.

In an *in vivo* study of mechanisms that might regulate RNA editing (Bhansali et al. 2007), it was found that infant maternal separation leads to increased RNA editing of the 5-HT_{2C} receptor as well as increased expression of $G\alpha_{q/11}$ protein in mouse cortex, suggesting that compensatory changes in $G\alpha_{q/11}$ expression occur in mice with persistently altered 5-HT_{2C} receptor RNA editing. However, a direct relationship seems unlikely given the current results. The alternative possibility that $G\alpha_q$ expression levels alter 5-HT_{2C} receptor RNA editing (Bhansali et al. 2007) was also not supported by the present findings, as the profile of 5-HT_{2C} receptor RNA editing was not altered in $G\alpha_q$ null mice.

Compensatory changes *in vivo*, other than alterations in the levels of $G\alpha_q$ proteins, that could maintain homeostatic signaling through differentially edited 5-HT_{2C} receptors include changes in the levels of expressed 5-HT_{2C} receptors (Barker & Sanders-Bush 1993). Indeed, recent findings show that INI/y mice expressing solely the non-edited and presumptively more active 5-$HT_{2C\text{-}INI}$ receptor have reduced expression of 5-HT_{2C} receptors in brain, whereas VGV/y mice expressing solely the fully-edited and presumptively less active 5-$HT_{2C\text{-}VGV}$ receptor have increased expression of 5-HT_{2C} receptors (Ronald Emeson, personal communication), findings confirmed in our laboratory (unpublished observations). Intracellular localization is markedly increased in recombinant cell lines expressing the highly functional INI receptor isoform (Marion *et al.* 2004, Chanrion et al. 2008), suggesting that receptor internalization may serve as an endogenous mechanism to maintain homeostatic signaling in cells expressing a relatively large proportion of highly active 5-HT_{2C} receptor isoforms. Conversely, increased surface expression of less active edited receptors may also be a mechanism to maintain homeostatic signaling.

Compensatory changes that may exist *in vivo* to maintain homeostatic signaling through differentially edited 5-HT_{2C} receptors are important given the clinical findings of increased editing of the 5-HT_{2C} receptor in the frontal cortex of suicide victims (Dracheva *et al.* 2007, Niswender *et al.* 2001, Gurevich *et al.* 2002, Iwamoto & Kato 2003). Although the present studies failed to find evidence for a compensatory relation between $G\alpha_{q/11}$ protein expression and 5-HT_{2C} receptor RNA editing, additional studies of adaptations in downstream signaling nodes are needed and may reveal novel targets for pharmacological interventions.

Abbreviations

5-HT_{2C}	serotonin 2C
WT	wild-type
PLCβ	phospholipase Cβ
IP3	inositol triphosphate
PIP2	phosphatidylinositol (4,5)-bis-phosphate

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DAG diacylglycerol

DOI 2,5-dimethoxy-4-iodoamphetamine

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Figure 1.
Dot plot of the mean percentage editing in Gαq null (−/−) and Gαq WT (+/+) mice for individual edit sites (top), RNA isoforms (middle), and predicted protein isoforms (bottom). This figure conveys at a glance the overall similarity in editing profiles between genotypes. Means and standard deviations for each genotype are tabulated in Table 2–Table 4, along with statistical test results. The figure also reveals that only 6 of 22 observed RNA isoforms were above 1% in frequency. In other words, relatively few isoforms captured most of the observed editing events.

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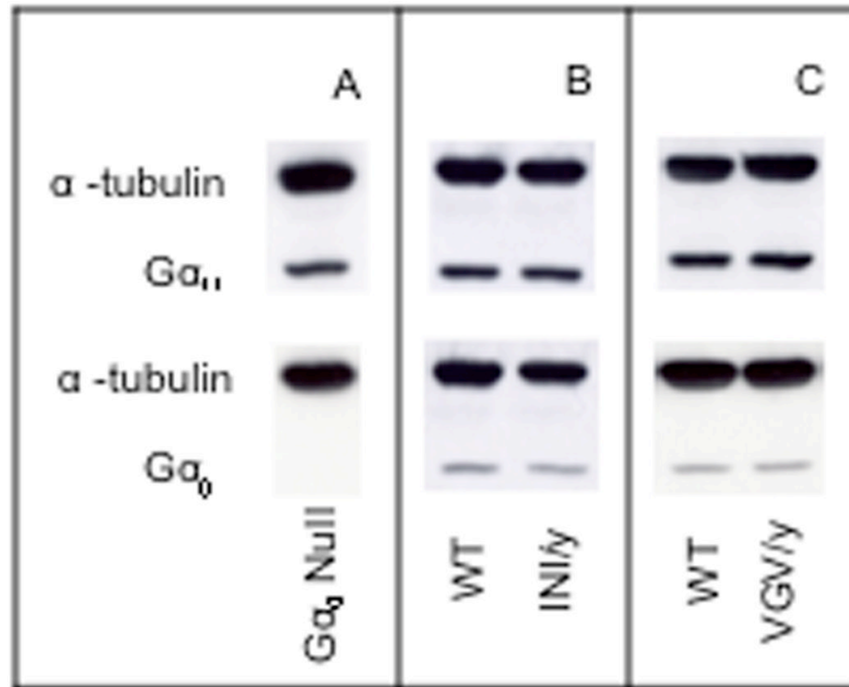

Figure 2.

(A) Western blots detecting $G\alpha_{11}$ at ~45 kDa (top) and $G\alpha_q$ at 45 kDa (bottom) and α-tubulin at 50 kDa (loading control) in cortical tissue of male $G\alpha_q$ null mice. Notice that $G\alpha_q$ protein was undetectable in null mice, yet $G\alpha_{11}$ protein expression was robust. (B) Western blots detecting $G\alpha_{11}$ (top) and $G\alpha_q$ (bottom) and α-tubulin (loading control) in cortical tissue of WT mice and mice expressing only the 5-$HT_{2C\text{-}INI}$ receptor isoform. (C) Western blots detecting $G\alpha_{11}$ (top) and $G\alpha_q$ (bottom) and α-tubulin (loading control) in cortical tissue from WT mice and mice expressing only the 5-$HT_{2C\text{-}VGV}$ receptor isoform.

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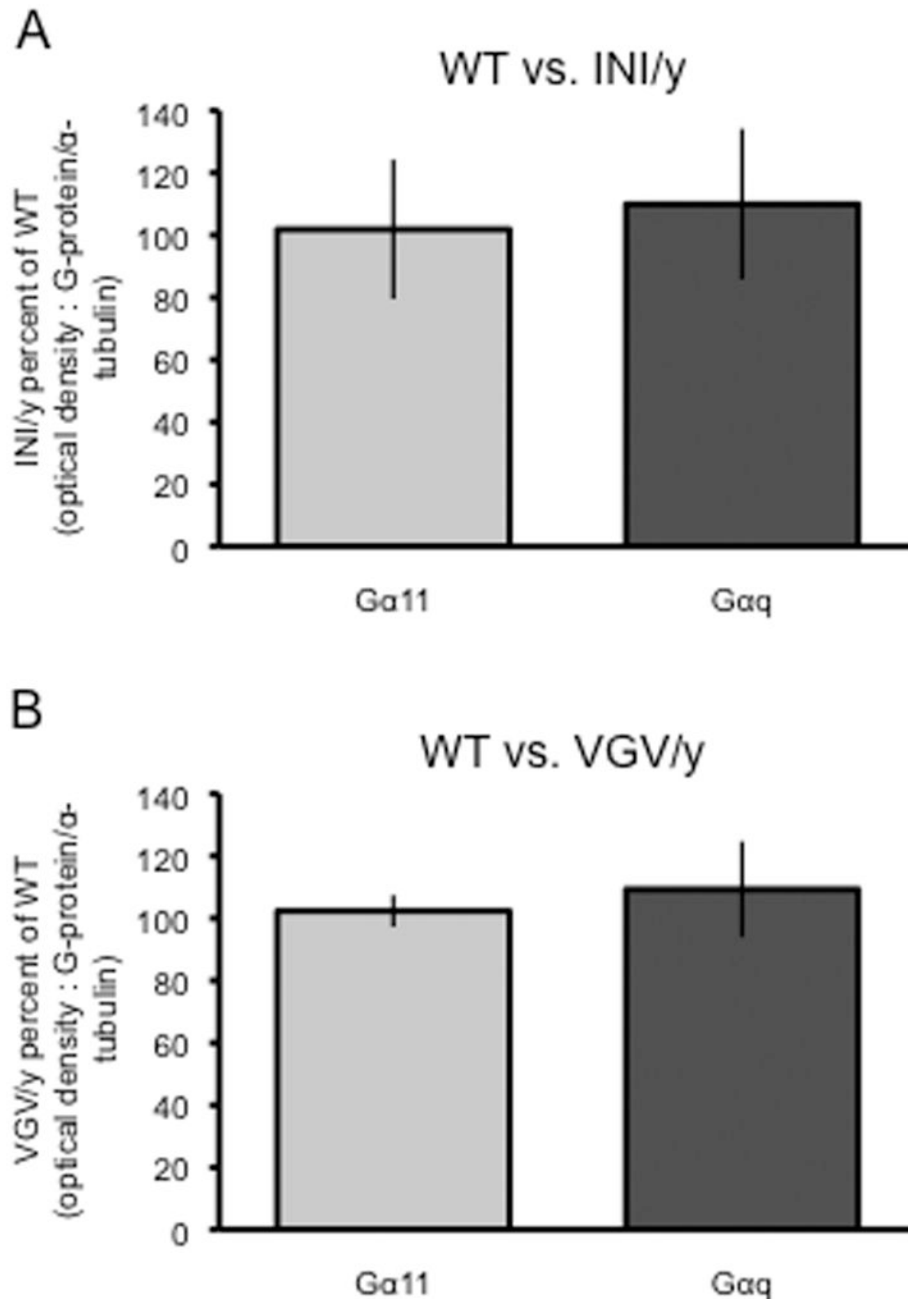

Figure 3.

(A) The mean optical density ratios of $G\alpha_{11}$ and $G\alpha_q$ to α-tubulin from Western blots of VGV/y cortical tissue are compared to the mean optical density ratios from Western blots in cortical tissue from wild-type mice. (B) The mean optical density ratios of $G\alpha_{11}$ and $G\alpha_q$ to α-tubulin from Western blots of INI/y cortical tissue are compared to the mean optical density ratios from Western blots in cortical tissue from wild-type mice. Unpaired t-tests revealed no significant differences in the levels of $G\alpha_{11}$ or $G\alpha_q$ in cortex of VGV/y ($p = 0.94$ and $p = 0.72$, respectively) or INI/y ($p = 0.77$ and $p = 0.56$, respectively) mice compared to their respective wild-type litter mates.

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Table 1

Translation between RNA editing at 5 sites A, B, E, C, and D and the predicted amino acids. Potential edited sites are in the top row, while edits are in the leftmost column; altered amino acids fill the table. For example, editing at sites A, B, and C translates to a VSI isoform.

Editing at:	Editing Sites		
	A B	**E C**	**D**
No sites	I	N	I
A	V		
B	M		
A and B	V		
E		D	
C		S	
E and C		G	
D			V

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Table 2

Percentage editing in Gαq null (−/−) and Gαq WT (+/+) mice. Shown are the mean percentages for editing at 5 individual edit sites, A, B, E, C, and D with standard deviations in parentheses, and uncorrected probabilities for the difference between genotypes.

Editing sites	A	B	E	C	D
WT	92(4)	85(7)	5(4)	23(3)	66(5)
Null	96(4)	87(6)	2(2)	24(7)	62(8)
t-test	0.135	0.556	0.051	0.790	0.282

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Table 3

RNA isoforms in Gαq null (−/−) and Gαq WT (+/+) mice. Shown are the mean percentages for 8 RNA isoforms with greater than 1% frequency with standard deviations in parentheses, and uncorrected probabilities for the difference between genotypes.

RNA isoform	A	AB	ABC	ABCD	ABD	AD	D	U
WT	4(3)	17(7)	7(1)	13(3)	44(5)	2(2)	3(1)	3(3)
Null	6(4)	19(6)	12(5)	10(5)	45(6)	2(2)	2(3)	1(2)
t-test	0.427	0.684	0.047	0.359	0.806	0.935	0.389	0.798

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Table 4

Predicted protein isoforms in Gαq null (−/−) and Gαq WT (+/+) mice. Shown are the mean percentages for 6 predicted protein isoforms greater than 1% frequency, with standard deviations in parentheses, and uncorrected probabilities for the difference between genotypes.

Protein isoform	INI	INV	VNI	VNV	VSI	VSV
WT	3(3)	3(1)	21(7)	47(7)	7(1)	13(3)
Null	1(2)	2(3)	24(7)	47(7)	12(4)	10(5)
t-test	0.404	0.389	0.452	0.856	0.016	0.303